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DOI: https://doi.org/10.1161/CIRCRESAHA.120.317721

Circulation Research

EDITORIAL

LDL Contributes to Reverse Cholesterol Transport

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To explain the atherogenicity and the antiatherogenicity of LDLs (low-density lipoproteins) and HDLs (high-density lipoproteins), respectively, many students and patients are taught that LDLs deliver cholesterol from liver to peripheral cells, whereas HDLs return excess cholesterol from peripheral cells back to liver for biliary excretion by reverse cholesterol transport (RCT). This keep-it-simple scheme is misleading. First, the vast majority of LDL cholesterol is taken up by the liver rather than by nonhepatic peripheral cells. Most of the latter can synthesize enough cholesterol for their need and limit the uptake of cholesterol with LDL by downregulation of *LDLR* (LDL receptor) expression. Only cells expressing nonsterol regulated scavenger receptors, notably macrophages, for example, in the atherosclerotic plaque, can take up excessive amounts of cholesterol via modified LDL.[1] Second, a large proportion of cholesterol carried by LDL is derived from HDL. The exchange of triglycerides carried by VLDLs (very-low-density lipoproteins) and their remnants against cholesteryl esters carried by HDL through the action of CETP (cholesteryl ester transfer protein) is an important source of LDL cholesterol. By CETP-mediated receipt of cholesteryl esters from HDL for subsequent LDLR-mediated uptake into the liver, LDL contributes to RCT. The blockage of this pathway may be one reason for the futility of CETP inhibitors toward prevention of major cardiovascular events, especially when combined with statins that upregulate LDLR and thereby enhance hepatic removal of LDL cholesterol.[2]

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In the current issue of *Circulation Research*, Cedo et al[3] highlight the importance of an additional and less well-known mechanism, by which LDL and the LDLR pathway contribute to RCT, namely by enhancing the flux of unesterified cholesterol (UC) from macrophages to the liver for fecal excretion. By cell culture experiments, they reproduced the findings of several laboratories that the presence of LDL increases the ability of plasma, native and reconstituted HDL, apoA-I, or albumin to promote efflux of UC from fibroblasts or different macrophage cell lines.[4–6] Cedo et al extend this previous knowledge by investigating the role of ABCA1 (ATP binding cassette transporter A1) and ABCG1 (ATP binding casette transporter G1). They report that LDL enhances efflux of UC directly with the help of ABCG1 and indirectly by serving as a sink for UC initially released from cells by HDL or lipid-free apoA-I through ABCG1 or ABCA1[3]. Their experiments like the previous studies[4–6] found that HDL shuttles UC very rapidly to LDL. The relative concentrations of HDL and LDL define the capacity of LDL to serve as an acceptor of cellular UC. The lower the concentration of HDL or the higher the concentration of LDL in the cell culture medium, the more cell-derived UC occurs in LDL. ApoB (apolipoprotein B)-containing lipoproteins were found to contribute about 40% to the cholesterol efflux capacity (CEC) of normolipidemic plasma but 65% to 85% to the CEC of plasmas from patients with HDL deficiency.[7] In the present study, plasmas of patients with familial hypercholesterolemia and plasmas of HAPOB Tg (transgenic for human ApoB) mice contained larger proportions of cell-derived UC in LDL than control plasmas from normolipidemic subjects and wild-type mice, respectively. This translated into increased

Key Words: Editorials ■ cholesterol esters ■ medical futility ■ plaque, atherosclerotic ■ triglycerides

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CEC for plasmas of HAPOB Tg mice.[3] Conversely, LDL apheresis decreases CEC of total plasma.[8] Interestingly and in contrast to LDL, VLDL does not support HDL in promoting cholesterol efflux from cells. Rather by contrast, VLDL inhibited HDL-induced cholesterol efflux, possibly by sterical hindrance of HDL/ABC transporter interaction.[3] An alternative explanation may be the recently demonstrated role of VLDL as a donor of UC to HDL.[9] Possibly, the UC transfer from VLDL to HDL competes with the UC transfer from cells to HDL.

LDL is not a permanent sink of cell-derived UC but distributes and traffics it for further metabolism. Cell-derived UC rapidly accumulating in LDL is slowly redistributed to HDL for esterification by lecithin:cholesterol acyltransferase.[4] Removal of LDL impairs the activity of plasma to esterify cell-derived UC.[4] Cedo et al[3] now show in mice that LDL and LDLR limit the reverse transport of radioactive UC from macrophages, installed either in the peritoneum or in the skin, to the feces. Knockout of *Ldlr* and enhanced LDLR degradation by overexpression of *PCSK9* (proprotein convertase subtilisin/kexin type 9) led to both the accumulation of macrophage-derived UC in LDL and decreased excretion of macrophage-derived UC with the feces.[3] These findings generally support the hypothesis that LDL and LDLR are important contributors to RCT, even in the absence of CETP, which is not expressed in mice.[2,10] However, the livers of neither *Ldlr*$^{-/-}$ mice nor *Ldlr*$^{-/-}$*HAPOB Tg mice nor mice overexpressing *PCSK9* contained less radioactive cholesterol as compared with wild-type control mice. These data do not contradict the general conclusion that LDL and LDLR limit RCT in mice but raise the question on alternative pathways for fecal cholesterol excretion, for example, transintestinal cholesterol excretion, which also involves LDL and is inhibited by PCSK9.[11]

The murine in vitro and in vivo models raise questions on the applicability of the data to humans.[10] For example, SR-BI (scavenger receptor BI) makes a larger contribution to cholesterol efflux from human macrophages than from murine macrophages.[12] As the consequence of CETP deficiency, selective uptake of HDL cholesterol through SR-BI is the major route of concluding RCT in mice.[10] In humans, CETP-mediated transfer of cholesteryl esters from HDL to LDL and subsequent LDLR-mediated LDL uptake eliminates at least half of HDL cholesterol from the circulation.[10] Although the UC transfer from HDL to LDL does not involve CETP, the predominance of the LDLR pathway in humans suggests that the findings in mice may underestimate its importance for RCT in humans. Conversely, the relevance of transintestinal cholesterol excretion for human cholesterol homeostasis is little understood.[11]

With respect to atherosclerosis, it is important to note that cholesterol efflux from macrophage foam cells happens in the arterial intima rather than in the plasma of the blood stream. According to our nowadays understanding of atherosclerosis, LDL does not leave the arterial wall but is retained in the extracellular matrix of the arterial intima by binding to proteoglycans, modified, and taken up by macrophages.[1] By contrast, HDL that has entered the arterial wall and eventually been loaded with macrophage-derived cholesterol can leave the arterial wall, probably via lymphatics.[13] Within the LDL sequestering environment of the arterial wall, the rapid transfer of UC from HDL to LDL may hence rather promote the accumulation of extracellular cholesterol rather than accelerate RCT. However, the transfer of both unesterified and esterified cholesterol from HDL to LDL for RCT may have occurred in evolution to relieve potentially dangerous situations of cellular cholesterol overload other than atherosclerosis, for example, caused by the clearance of cells in the reticulohistiocytic system or lipolysis in adipose tissue during prolonged fasting.

Many clinical and epidemiological studies measured CEC of ApoB-depleted serum or plasma as surrogates of HDL functionality and found it associated with the presence or incidence of atherosclerotic cardiovascular disease.[12,14] However, despite increasing CEC, CETP inhibitors did not reduce major cardiovascular event rates in randomized controlled trials.[2,12] The strong impact of LDL on CEC of plasma raises the question whether the use of total rather than ApoB-depleted serum or plasma improves the predictive value of CEC. Only one study compared the 2 specimens toward their prognostic performance. In this study,[15] CEC of total serum but not CEC of ApoB-depleted serum was associated with all-cause mortality independently of HDL cholesterol and other risk factors.

In conclusion, by using state-of-the-art experimental models, Cedo et al[3] confirm that LDL amplifies the cholesterol efflux mediated by HDL. They extend previous knowledge by unraveling the contribution of ABCA1 and ABCG1 and by showing the in vivo relevance of LDL and LDLR for RCT of macrophage-derived cholesterol. The relevance of these findings for human (patho)physiology, especially atherosclerosis, needs to be shown. However, they should have impact on the design of assays to determine CEC.

ARTICLE INFORMATION

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Disclosures

None.

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